Electronic Supplementary Material (ESI) for Polymer Chemistry.

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Electronic Supplementary Information for

Novel target NIR-fluorescent polymer for living tumor cell imaging

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Materials

Dichloromethane (DCM), N,N-dimethylmethanamide (DMF) triethylamine (TEA) Methyl methacrylate (MMA, Adamas) were heated at reflux with CaH_2 and then distilled prior to use. 2,2-Azobis(isobutyronitrile) (AIBN 98%) was recrystallized twice from ethanol and then dried at 25°C for 2 days in a vacuum over before use. D-(+)-Glucose (99%, Adamas) Methacryloylchloride (95%) was purchased from Adamas. 1-Propanethiol (99%) was obtained from Adamas. Boron trifluoride etherate(98%, Aladdin), 2-(4-Hydroxybenzal)Acetophenone(97%, Alfa) , Nitromethane (99% , Adamas), 2-Bromoethanol(96% , Adamas), Diethylamine(99% , Adamas), N,N,N,N',N'-Pentamethyldiethylenetriamine(PMDETA, 98% , Aladdin, Ammonium acetate(98% , Adamas) , N-Ethyldiisopropylamine (DIEA, 99% , Adamas),WZB117(99%, Sigma). Unless mentioned, all other materials and reagents were commercially purchased and used without any purification.

Measurements

Both ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Varian MERCURY plus 400 NMR spectrometer at 298 K using deuterated chloroform ($CDCl_3$) or dimethyl sulfoxide-d_6 (DMSO-d_6) and Methanol-d_4 (CD_3OD-d_4)as the solvent. The chemical shifts were referenced to residual peaks of deuterated solvents: $CDCl_3$ (7.26 ppm); DMSO-d_6 (2.48 ppm); CD_3OD-d_4 (3.31 ppm). Fourier transform infrared (IR) spectra were tested using a PerkinElmer Paragon 1000 spectrophotometer between 4000 and 450 cm^{-1}. All sample pellets were prepared by grinding the solid sample with dry potassium bromide (KBr) under high pressure.

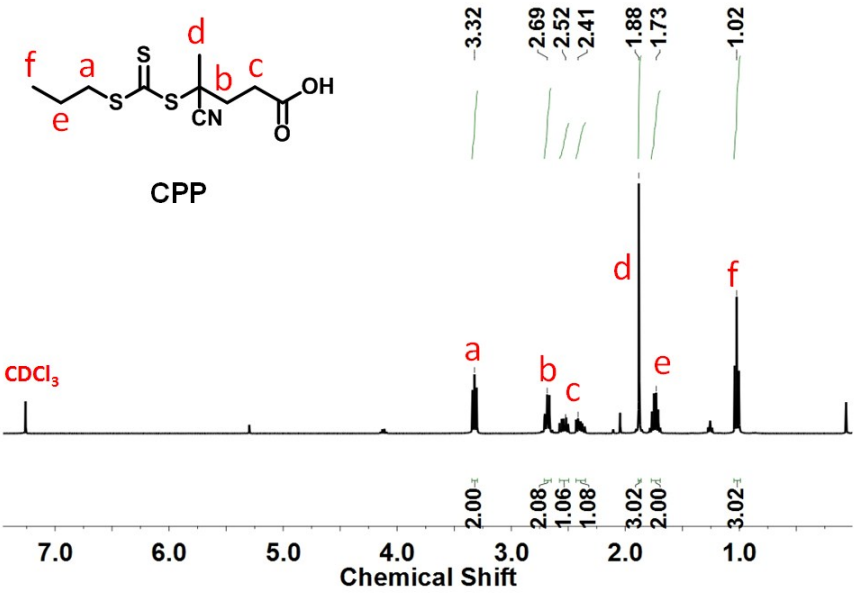
High-resolution mass spectrometry (HRMS) data were obtained for each sample from 50 to 1000 Da with a 0.10 s scan time and a 0.01 s interscan delay over a 10 min analysis time on a Waters Micromass Q-TOF Premier mass spectrometer. The molecular weights and polydispersity index (PDI) were measured by gel permeation chromatography EcoSEC (ECS000113). Tetrahydrofuran (THF) was used as the mobile phase at a flow rate of 1 mL/min at 30°C. Transmission electron microscopy (TEM) images were obtained using a JEOL JEM-100CX-II instrument at a voltage of 200 kV. Samples were prepared by drop-casting solution onto carbon-coated copper grids and then freeze-drying under vacuum before measurements. Dynamic light scattering (DLS) measurements were carried out on a Malvern Zetasizer Nano ZS90 apparatus equipped with a 4.0 mW He−Ne laser operating at $\lambda = 633$ nm. All samples were measured at room temperature with a scattering angle of 90°. The fluorescence emission measurement was measured on QM/TM/IM fluorescence Lifetime Spectrometer (PTI company) at room temperature. The bioimaging was measured on (Leica)/TCS SP8STED 3X Super-resolution Multiphoton Confocal Microscope. The flow cytometry experiment was measured on MoFlo XDP(Beckman Coulter).

Synthetic Procedures

Synthesis of Compound CPP

Compound CPP was prepared by a modified way of a previously published protocol,[1]1-Propanethiol (5.07 g, 67 mmol) was added dropwise to a solution of potassium hydroxide (4.67 g, 83 mmol) in 70 mL of water, followed by carbon disulfide (5.07 g, 67 mmol) in one portion. The resulting solution was vigorously

stirred at room temperature for 30 min and cooled to 0°C, p-Tosyl chloride (6.35 g, 33 mmol) in distilled acetone (100 mL) was added in portions over 10 min, and stirring was continued for 2 h. The acetone was then evaporated in an open vessel under stirring. Afterward, the red oil was extracted with dichloromethane; the organic layer was washed with water and dried over anhydrous $MgSO_4$ overnight. The $MgSO_4$ was filtered off, and dichloromethane was removed by rotary evaporation to yield solid bis(propylsulfanylthiocarbonyl) disulfide. A solution of 4,4′-azobis(4-cyanopentanoic acid) (8.55 g, 30.5 mmol) and bis(propylsulfanylthiocarbonyl) disulfide (8.36 g, 27.7 mmol) in ethyl acetate (100 mL) was heated under reflux for 20 h. After removal of the volatiles in vacuo, the crude product was purified by column chromatography on silica gel with a mobile phase of (diethyl ether/hexane =1/2) 5.8 g, yield: 75.5%.



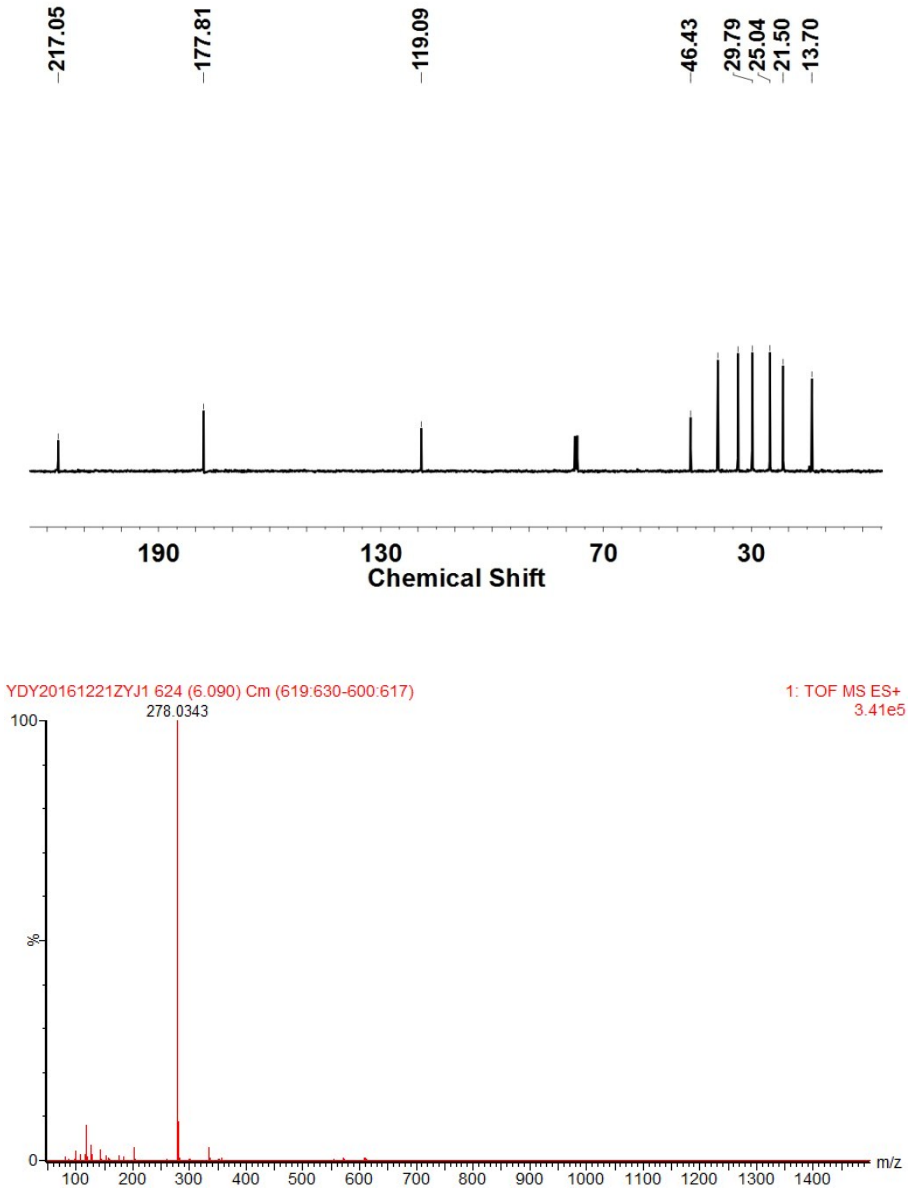
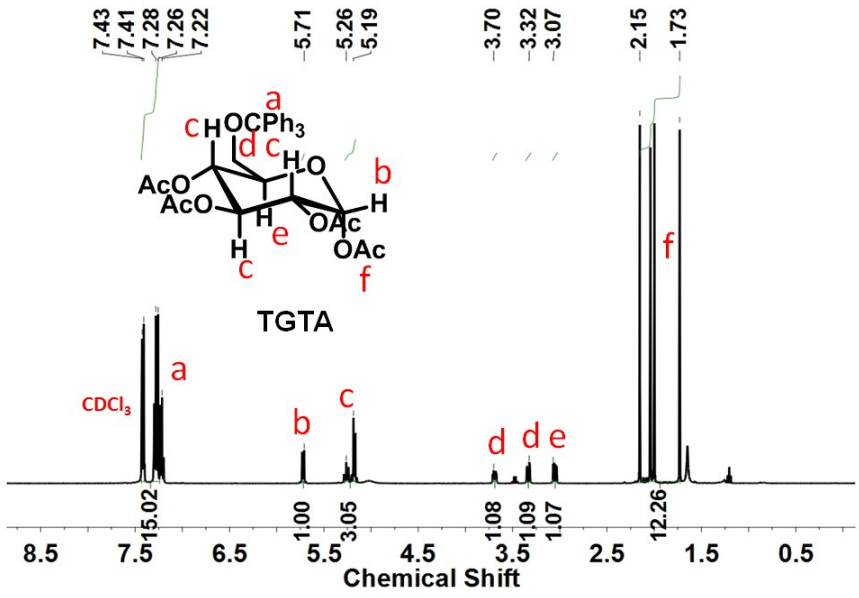


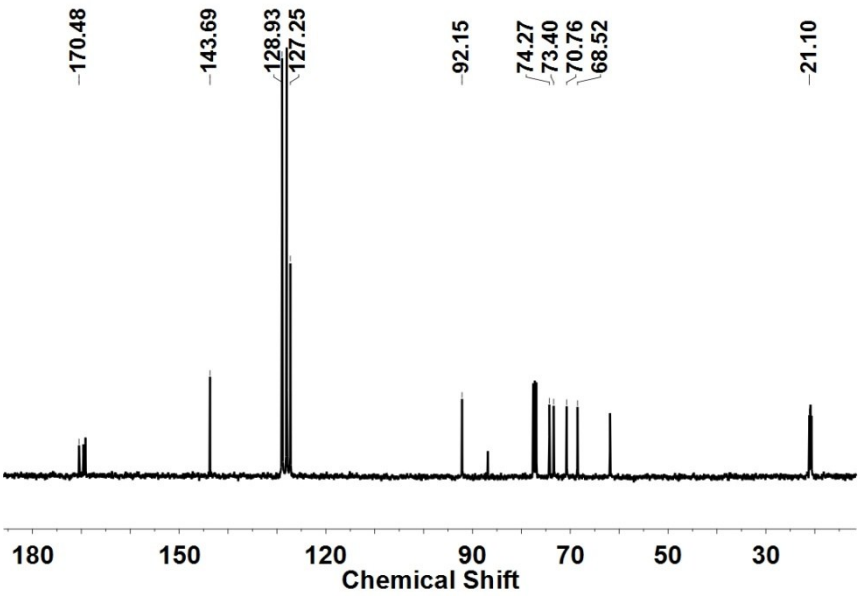
Figure S1. ^{1}H NMR ,^{13}C NMR spectra of the CPP in $CDCl_3$ and LC-MS.

Synthesis of Compound TGTA

Compound TGTA was prepared by a modified way of a previously published protocol,[2] D-(+)-Glucose (30 g, 167 mmol) and Triphenylmethyl Chloride (50 g, 180 mmol) was dissolved in anhydrous pyridine(150 mL) and the mixture was preheated in oil-bath to 90°C, until the D-(+)-Glucose was fully dissolved, acetic anhydride (90 mL) was added and allowed to stir at room temperature for 16 h. Afterward the

mixture was carefully poured into the mixture solution of ice water (4 L) and acetic acid (250 mL). The precipitate was dramatically stirred for 3 h. The white precipitate was filtered, and washed with cold water, after dried at room temperature conditions. The white solid obtained was dispersed in anhydrous ether (150 mL) and filtered to afford the solid, after vacuum drying, afford the white solid (40 g, yield: 40.6%).





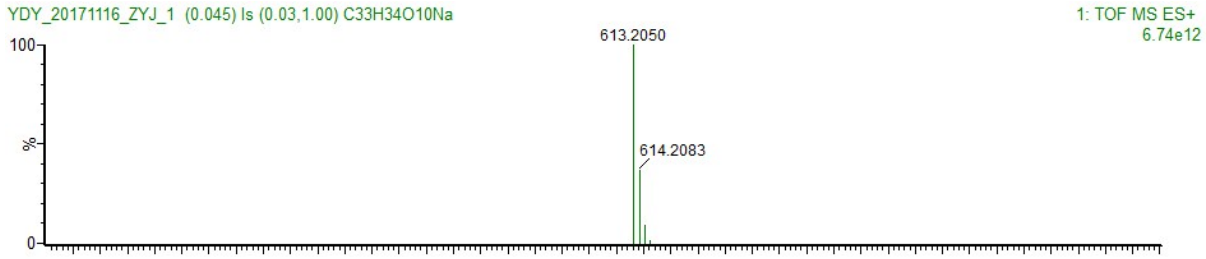
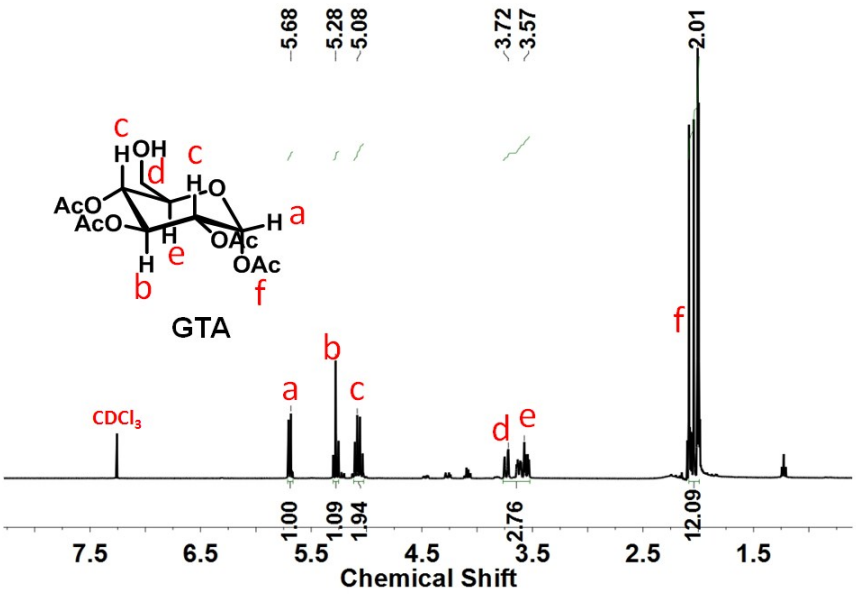


Figure S2. ^{1}H NMR ,^{13}C NMR spectra of the TGTA in $CDCl_3$ and HRMS.

Synthesis of Compound GTA

In a 250 mL reaction flask, compound TGTA (35 g, 58.4 mmol) was dissolved in 300 mL acetic acid, after heating to dissolve, acetic acid solution of hydrogen bromide (20 mL) was added via a drop-wise manner at ice bath. After stirring for 5 min, faint yellow solid (trityl bromide) appeared and was separated by filtration, the filtrate was poured into the cold water (1L), and extracted with chloroform, the solution was washed by ice water for 4 times, and dried with $MgSO_4$ to get the crude product, then purified by silica gel (Ethyl Acetate: Hexane = 2: 8) to afford 12 g white solid. Yield 59%.



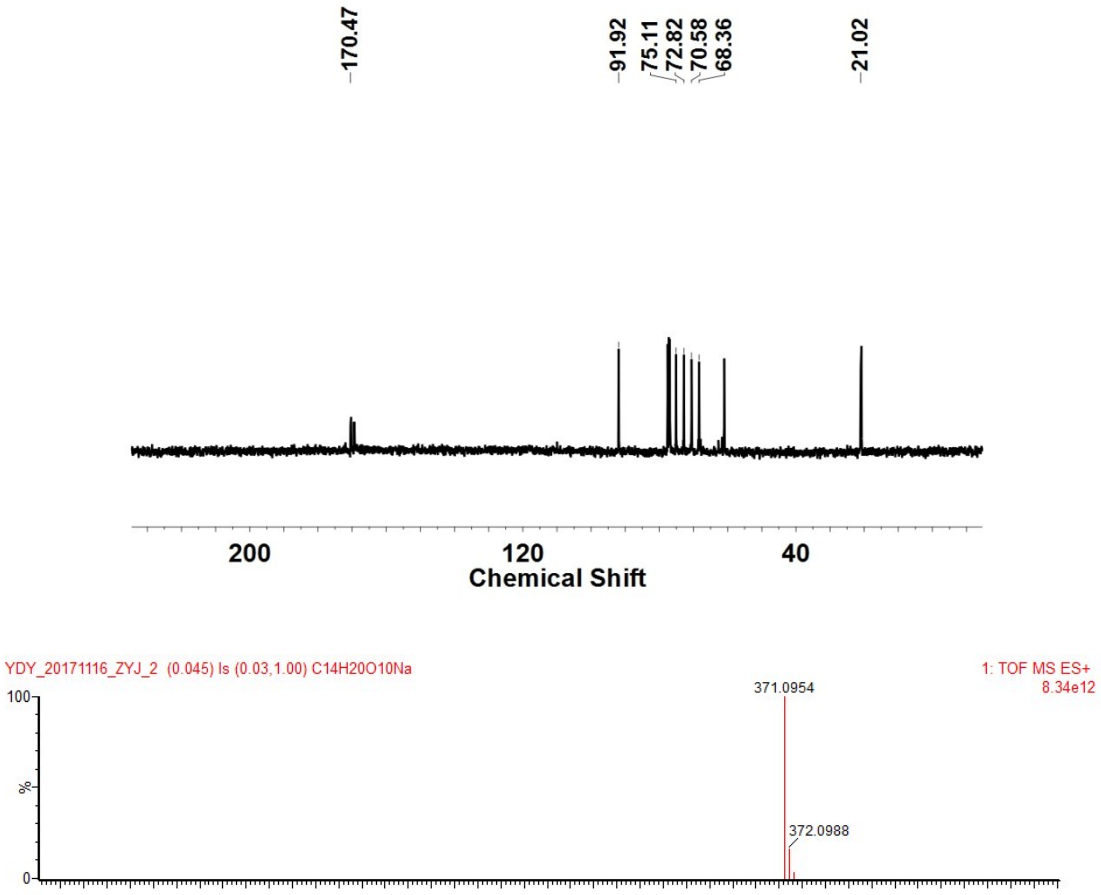


Figure S3. ^{1}H NMR and ^{13}C NMR spectra of the GTA in $CDCl_3$ and HRMS.

Synthesis of Compound GATA

In a 250 mL reaction flask, compound GTA (2.14 g, 6.42 mmol) was dissolved in 50 mL dry THF, then Et_3N (2.7 mL, 19.26 mmol) was added with stirring at ice bath. 20 mL dry THF including methacryloyl chloride(1.82 g, 17.33 mmol) was dropped in reaction flask under nitrogen protection, and continued to react for 24 h at room temperature. After ending, the mixture was filtered and the filtrate was washed by deionized water twice and dried with $MgSO_4$ to get the crude product, then purified by silica gel (Ethyl Acetate: Hexane=3: 7) to afford 2.09 g white solid. Yield 78.2%.

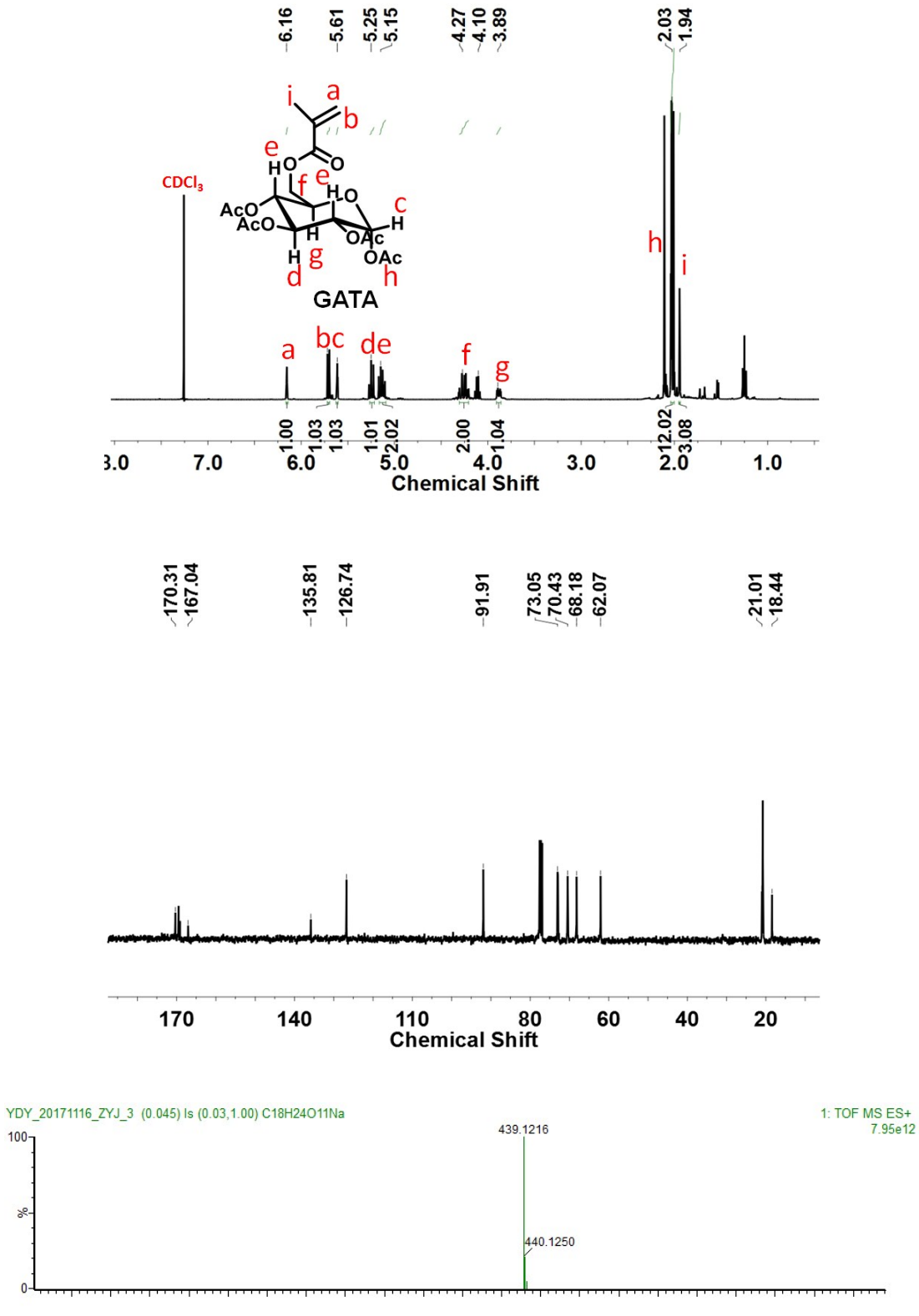
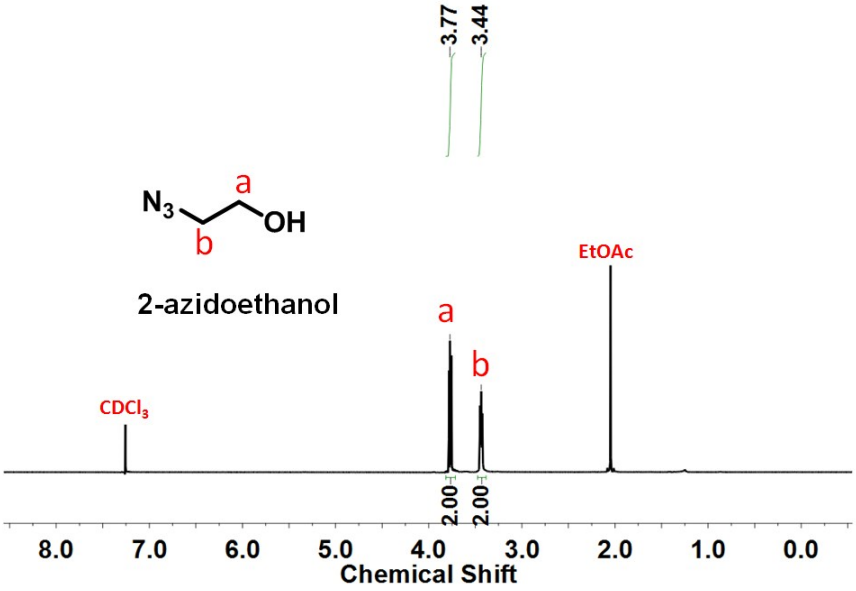


Figure S4. ^{1}H NMR and ^{13}C NMR spectra of the GATA in $CDCl_3$ and HRMS.

Synthesis of Compound 2-azidoethanol

Compound 2-azidoethanol was prepared by a modified way of a previously published protocol, [3] Sodium azide (13.75 g, 211.5 mmol) was dissolved in 150 mL deionized water, followed by adding 2-Bromoethanol (5 mL, 70.5 mmol) in one portion. The resulting solution was stirred at 80°C for 16 h, and cooled to room temperature, then extracted with anhydrous ether for three times, and dried over anhydrous $MgSO_4$, anhydrous ether was removed by rotary evaporation to yield colorless liquid 4.9 g, yield: 80%.



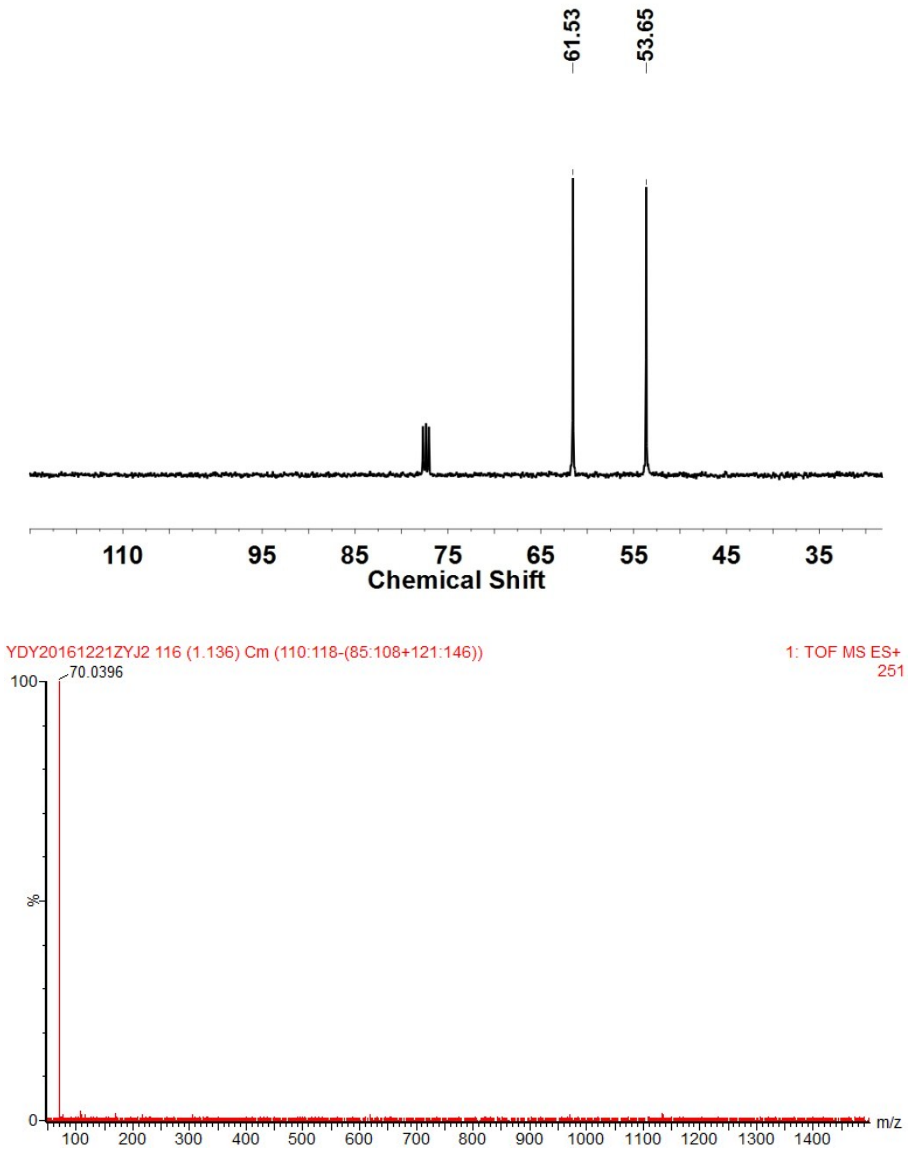
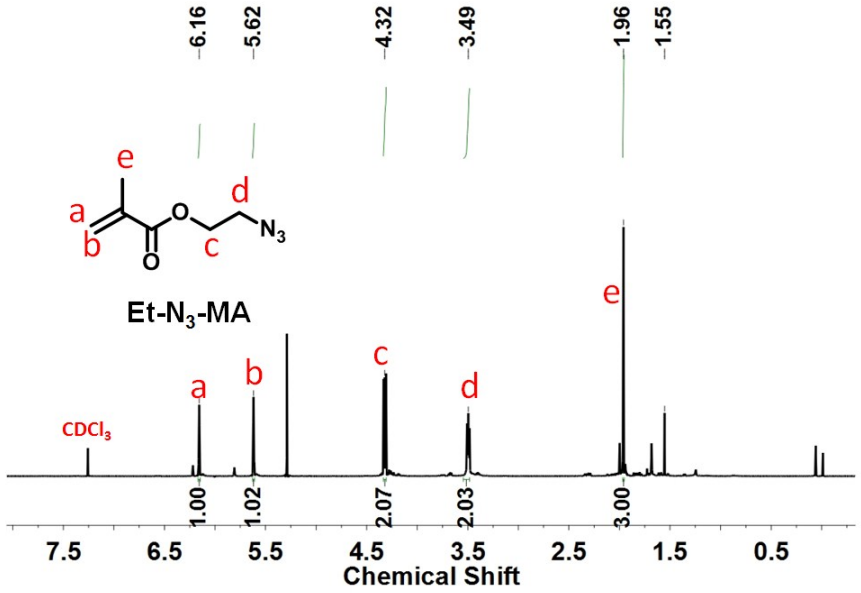


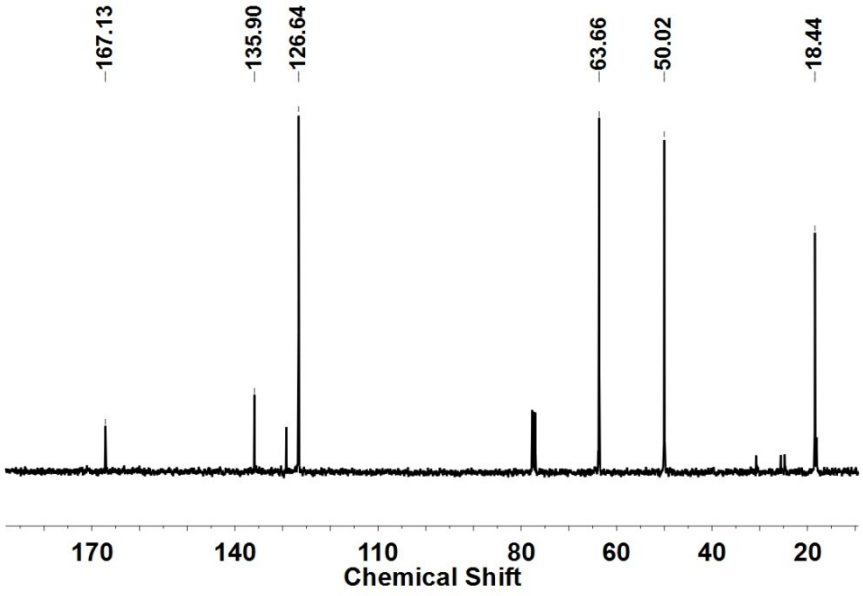
Figure S5. ^{1}H NMR and ^{13}C NMR spectra of the 2-azidoethanol in $CDCl_3$ and DMSO-d_6 and LC-MS.

Synthesis of Compound Et-N_3-MA

In a 250 mL reaction flask, compound 2-azidoethanol (3 g, 34.45 mmol) was dissolved in 50 mL dried CH_2Cl_2, then Et_3N (5.79 mL, 41.34 mmol) was added with stirring at ice bath. 20mL dry CH_2Cl_2 with methacryloyl chloride (4.32 g, 41.34 mmol) was dropped in reaction flask, and continued to react under nitrogen protection for 24 h at room temperature. After ending, The mixture was filtered and the filtrate was

washed successively by saturated $NaHCO_3$, saturated brines and deionized water, and dried with $MgSO_4$ to get the crude product, then purified by silica gel(dichloromethane: Hexane=3: 7) to afford 4.1 g colorless liquid. Yield 75%.





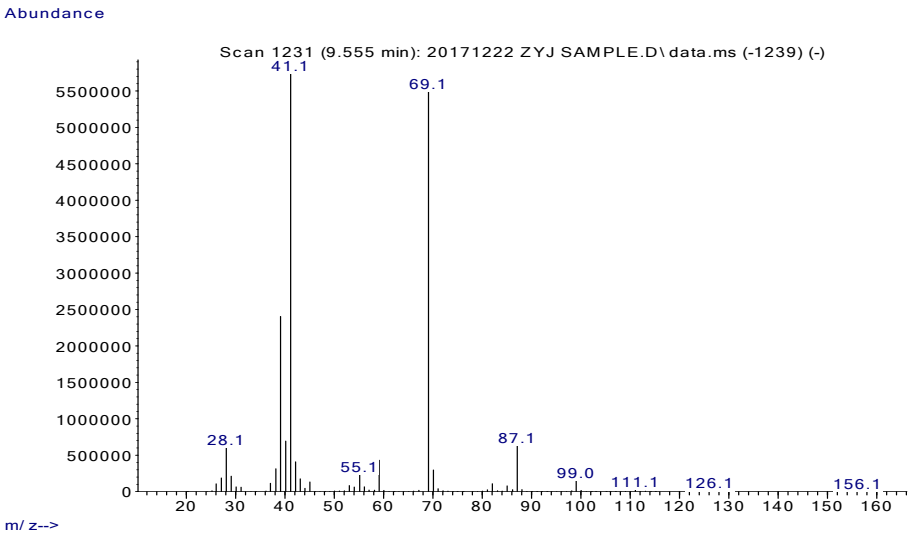
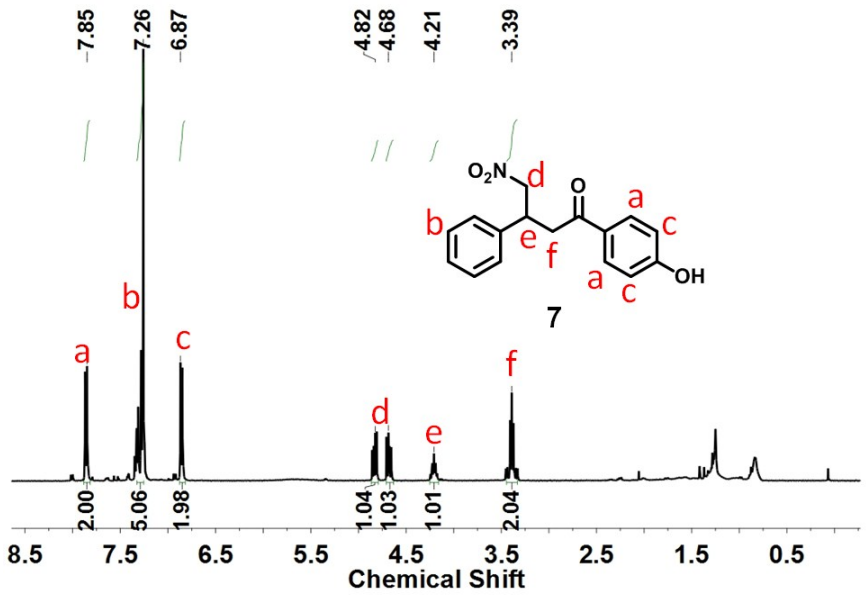
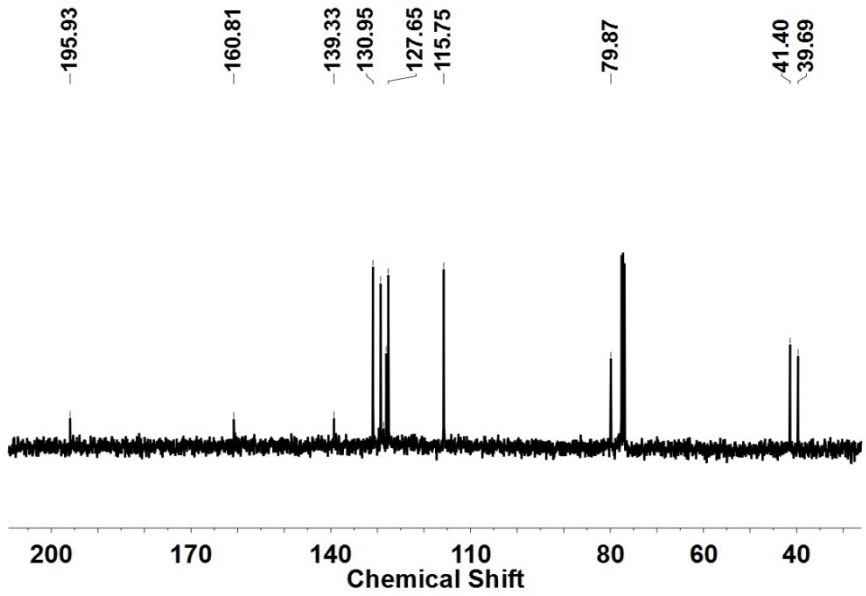


Figure S6. ^{1}H NMR and ^{13}C NMR spectra of the Et-N_3-MA in $CDCl_3$ and GC-MS.

Synthesis of Compound 7

Compound 7, 1-(4-Hydroxyphenyl)-4-nitro-3-phenylbutan-1-one was prepared by a modified way of a previously published protocol, [4] 1-(4-hydroxyphenyl)-3-phenylpropenone (3.2 g, 14.3 mmol), nitromethane (8.71 g, 142.8 mmol) and diethylamine (5.22 g, 71.3 mmol) was dissolved in 40 mL dried EtOH, and heated to reflux for 24 h, The solution was cooled to room temperature, and acidified with 1M HCl, the crude product was purified by column chromatography on silica gel with a mobile phase of (Ethyl Acetate /hexane =3:7) to afford 2.97 g faint yellow solid 2.97 g. Yield 72.8%.





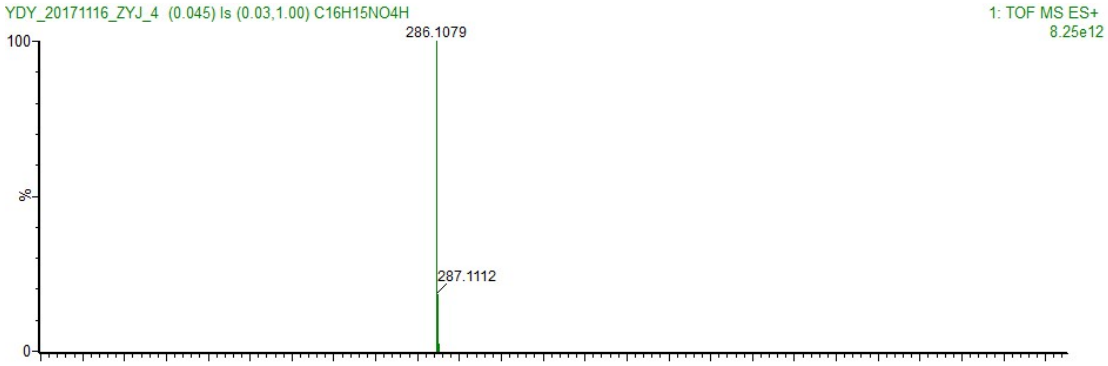
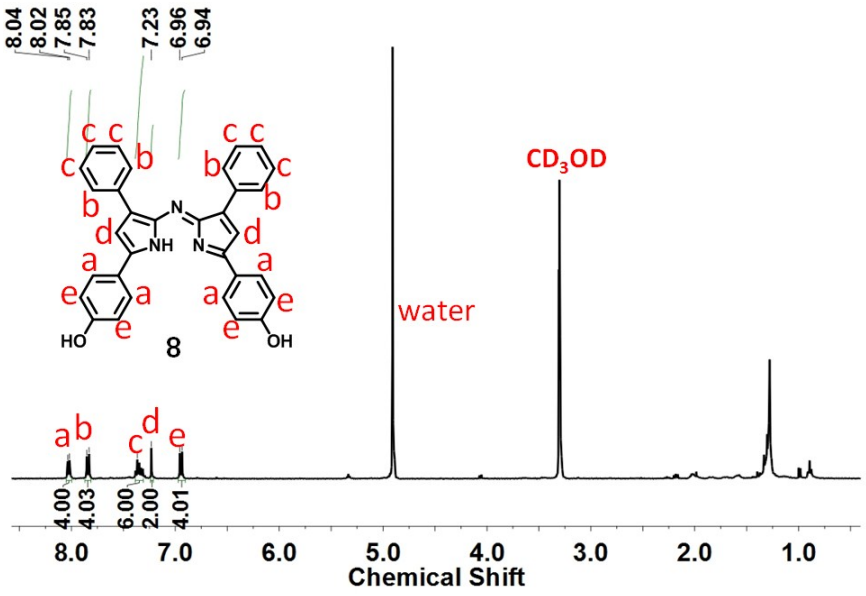


Figure S7. ^{1}H NMR and ^{13}C NMR spectra of the compound 7 in $CDCl_3$ and HRMS.

Synthesis of Compound 8 [5-(4-Hydroxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl)-3-phenylpyrrol-2-ylidene]amine

Compound 7 (1 g, 3.5 mmol), ammonium acetate (9.45 g, 122.5 mmol) was dissolved in 200 mL dried EtOH, and heated to reflux for 24 h, and black blue solid was formed under reacting. After ending, the solution was cooled to room temperature, and removed by rotary evaporation, then washed with deionized water for three times, filtered and dried under vacuum condition to obtain black solid 730 mg. Yield 43.3%.



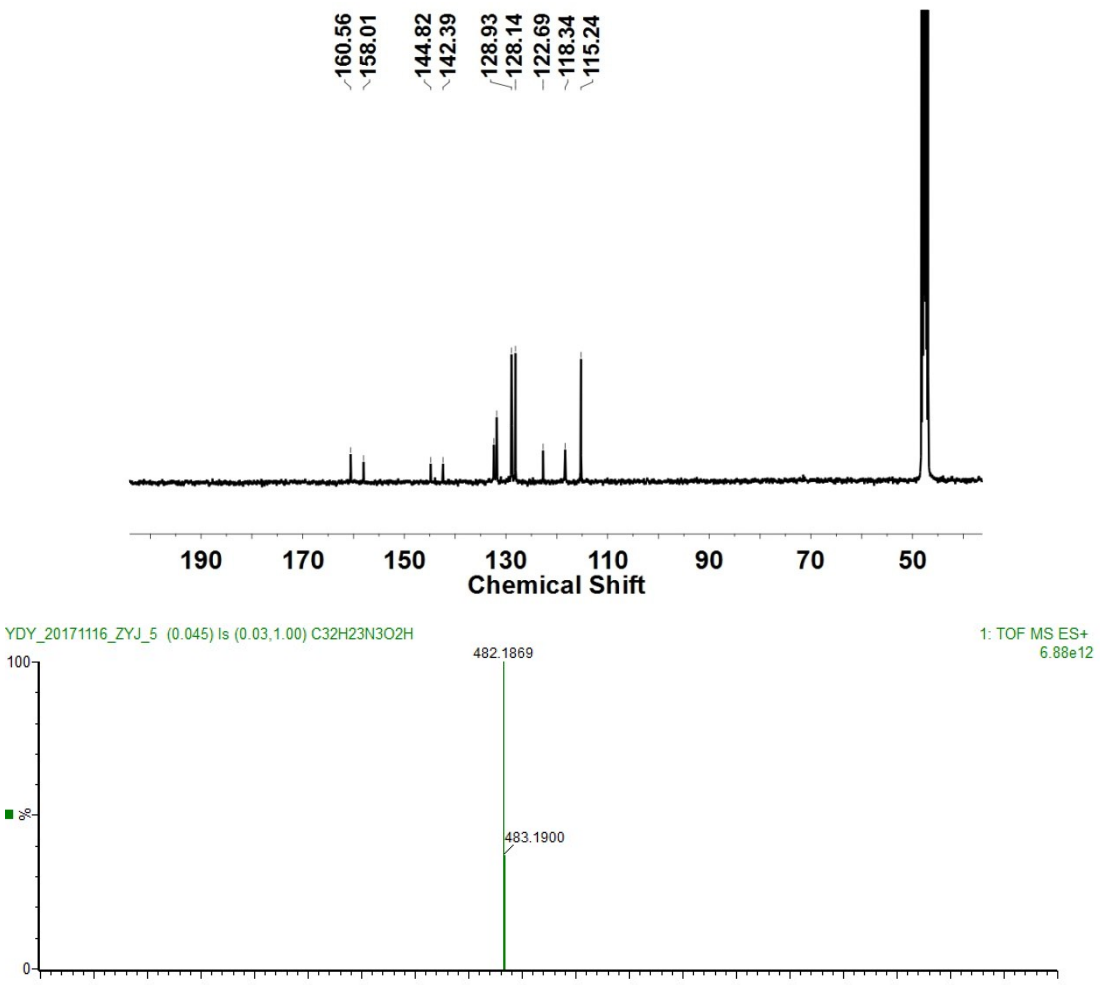


Figure S8. ^{1}H NMR and ^{13}C NMR spectra of the compound 8 in CD_3OD and HRMS.

Synthesis of Compound 9

BF_2 chelate of [5-(4-hydroxyphenyl)-3-phenyl-1H-pyrrol-2-yl]-[5-(4-hydroxyphenyl)-3-phenylpyrrol-2-ylidene]amine

Compound 8 (1 g, 3.5 mmol) was dissolved in 100 mL dried CH_2Cl_2, then DIEA (3.04 mL, 23.5 mmol) was added with stirring at ice bath. 20 mL dried CH_2Cl_2 with $BF_3{\cdot}OEt_2$ (4.2 mL, 33 mmol) was dropped in reaction flask under nitrogen protection for 24 h at room temperature. After ending, The mixture was washed successively by saturated $NaHCO_3$, saturated brines and deionized water, and dried with $MgSO_4$ to get the crude product, then purified by silica gel (ethyl acetate: hexane=4: 6) to afford

700 mg dark red solid. Yield 56.3%.

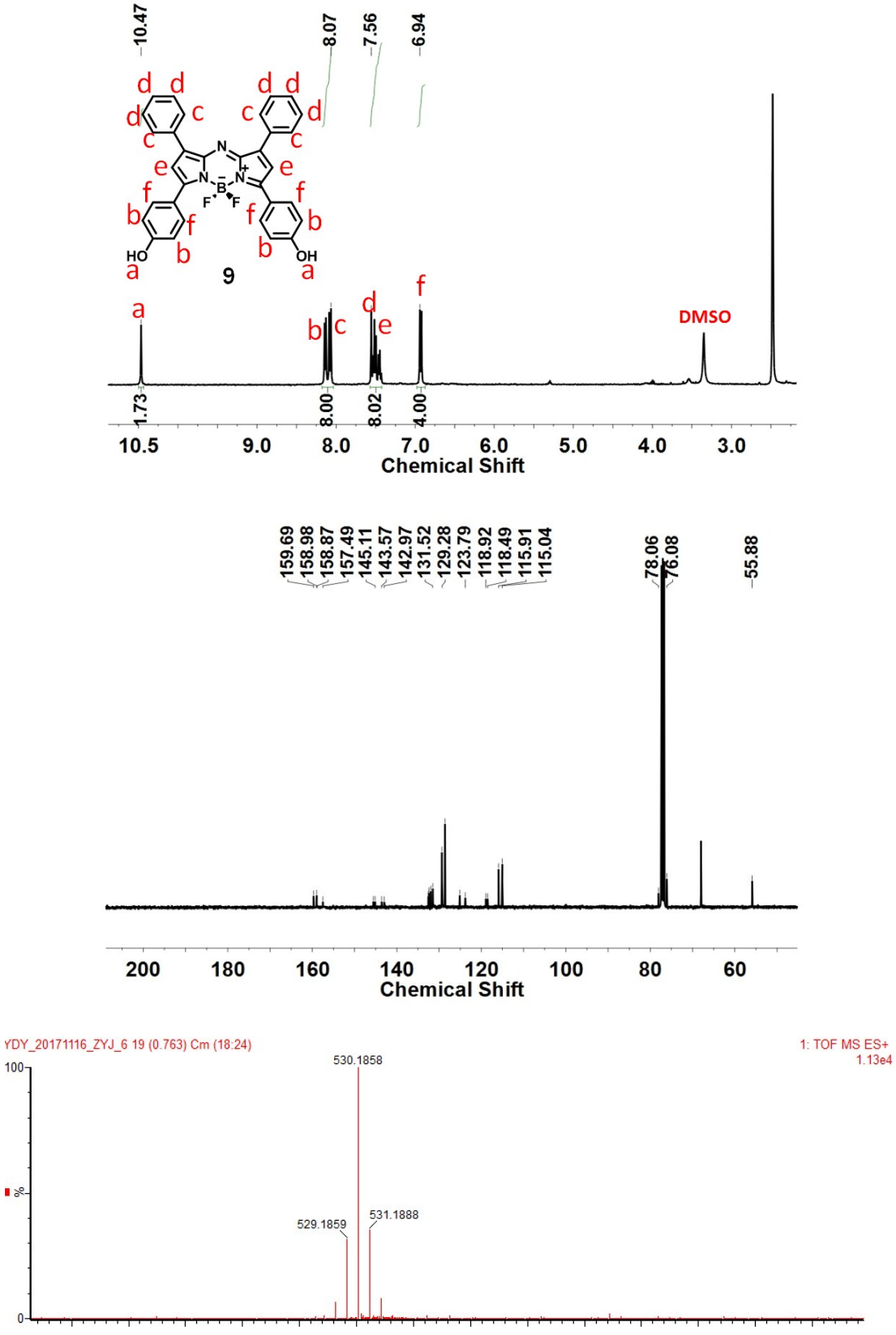


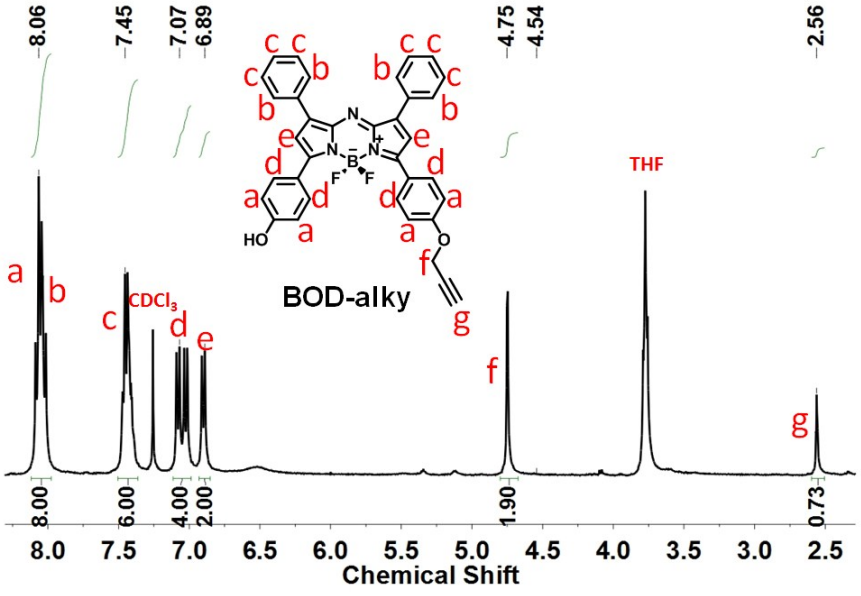
Figure S9. ^{1}H NMR and ^{13}C NMR spectra of the compound 9 in DMSO-d_6 and CD_3OD and

HRMS.

Synthesis of Compound 10

BF_2 chelate of 4-{4-phenyl-5-[3-phenyl-5-(4-prop-2-ynyloxyphenyl)-pyrrol-2-ylideneamino]-1H-pyrrol-2-yl}phenol

Compound 9 (100 mg, 0.189 mmol), NaH (16.87 mg, 0.703 mmol) was dissolved in 10 mL dried THF, then 5 mL dried THF solution with propargyl benzenesulfonate (87.46 mg, 0.416 mmol) was added dropwise in reaction flask under nitrogen protection for 2 h at ice bath. After heating to reflux for 6 h, the solution was cooled to room temperature, and extracted with ethyl acetate and saturated brines, the organic layer was washed by deionized water for three times, and dried with $MgSO_4$ to get the crude product, then purified by silica gel (ethyl acetate: hexane=3: 7) to afford 40 mg dark solid. Yield 37.3%.



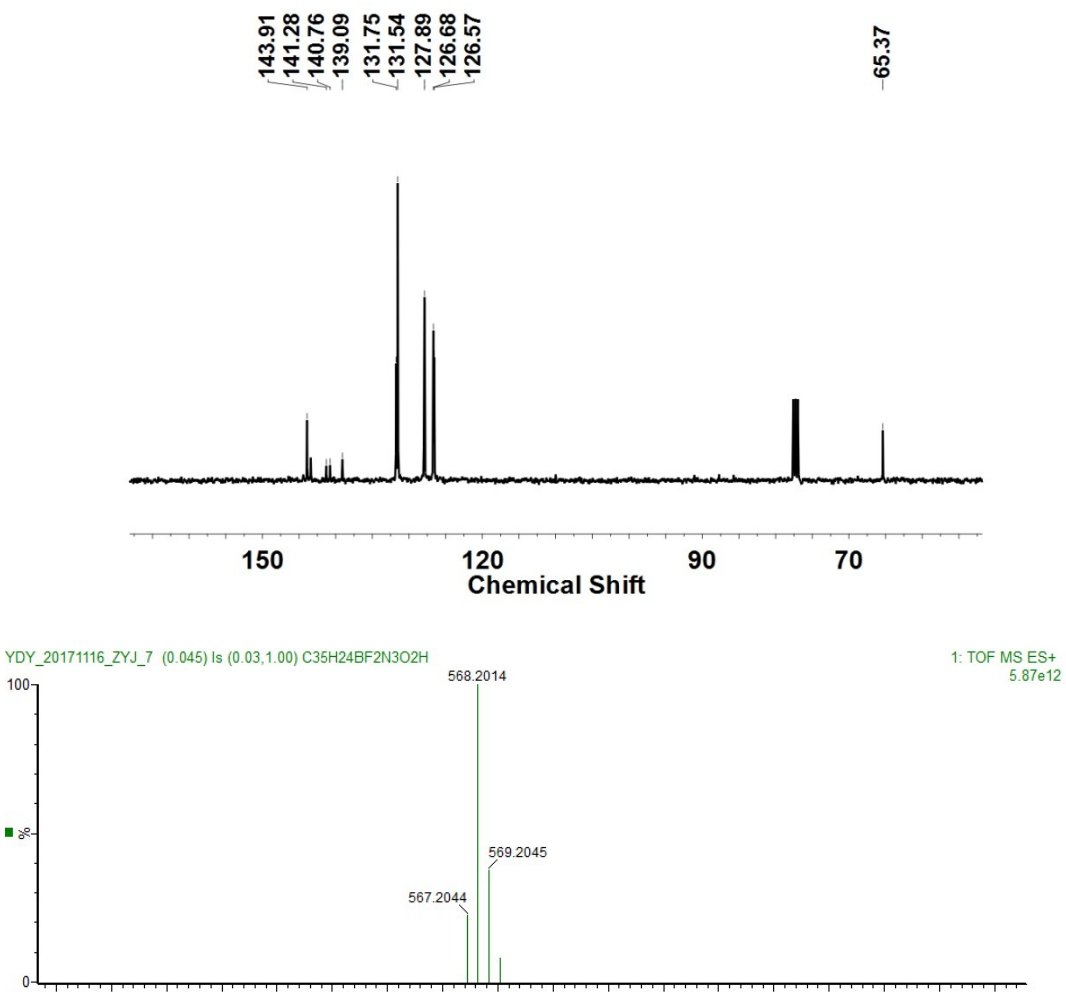


Figure S10. ^{1}H NMR and ^{13}C NMR spectra of the compound BOD-alky in $CDCl_3$ and HRMS.

Synthesis of PMMA

PMMA was prepared by RAFT copolymerization. MMA (3 mL, 28.29 mmol), CPP (39.1 mg, 0.141 mmol), and AIBN (2.3 mg, 0.014 mmol) were added to a round-bottom flask with a magnetic stirring bar, and DMF (5 mL) was added as solvent. The flask was sealed and the solution was bubbled with nitrogen for 30 min. Subsequently, the flask was immersed into 70°C oil-bath. After reaction for 20 h under nitrogen atmosphere, the solution was quenched by liquid nitrogen. The residue was precipitated into cold diethyl ether for three times and then the product was purified and dried under vacuum overnight. The purified white product 1.8 g was obtained.

Synthesis of Compound PMMA-b-P(GATA-co-N_3)

PMMA-*b*-P(GATA-*co*-N_3) was prepared by RAFT copolymerization. PMMA (510 mg, 0.033 mmol), GATA (343.5 mg, 0.825 mmol), 2-azidoethyl methacrylate (10.4 mg, 0.066 mmol), and AIBN (0.54 mg, 0.0033 mmol) were added to a round-bottom flask with a magnetic stirring bar, and DMF (5 mL) was added as solvent. The flask was sealed and the solution was bubbled with nitrogen for 30 min. Subsequently, the flask was immersed into 70°C oil-bath. After reaction for 20 h under nitrogen atmosphere, the solution was quenched by liquid nitrogen. The residue was precipitated into cold diethyl ether for three times and then the product was purified and dried under vacuum overnight. The purified white products 675 mg were obtained.

Synthesis of PMMA-b-P(GATA-co-BOD)

PMMA-*b*-P(GATA-*co*-BOD) was prepared by click reaction. PMMA-*b*-P(GATA-*co*-N_3) (308 mg, 0.016 mmol), BOD-alke (22.6 mg, 0.04 mmol), PMDETA (6.72 μL, 0.032 mmol) and CuCl (3.2 mg, 0.032 mmol) were added to a round-bottom flask with a magnetic stirring bar, and DMF (5 mL) was added as solvent. The flask was sealed and the solution was bubbled with nitrogen for 30 min. Subsequently, the flask was immersed into 50°C oil-bath. After reaction for 24 h under nitrogen atmosphere, the solution was quenched by liquid nitrogen, the mixture was diluted by 15 mL THF, and passed through a short neutral alumina column to remove copper catalysts. The residue was precipitated into cold diethyl ether for three times and then the product was purified and dried under vacuum overnight. The purified green products 280 mg

were obtained.

Deprotection of PMMA-b-P(GATA-co-BOD)

In a 10 mL reaction flask, polymer compound 13 (100 mg, 0.005 mmol) was dissolved in 5 mL THF, then added dropwise 1.5 mL methyl alcohol solution of $NaOCH_3$, stirring at room temperature for 3 h. After ending, the mixture was neutralized with 4M HCl until to neutral. The residue was dialyzed in distilled water for 3 day, and lyophilized to obtain a green powder of product 80 mg.

Preparation of Polymer Nanoparticles

PMMA-*b*-P(GATH-*co*-BOD) nanoparticles were obtained by dialysis method. The polymer (2 mg) was dissolved in 1mL THF, and subsequently the polymer solution was dialyzed against deionized water for 24 h (MWCO = 1 kDa), and the deionized water was replaced every 4 h.

Cell Culture

MCF-7 lines (Michigan Cancer Foundation-7 cell line) were cultured with DMEM media include 10% fetal bovine serum (FBS), 50 units/mL antibiotics penicillin and streptomycin in a 5% CO_2 cell incubator at 37°C. RWPE-1 line (Normal prostate epithelial cells) were cultured with K-SFM media without fetal bovine serum (FBS), 50 units/mL antibiotics penicillin and streptomycin in a 5% CO_2 cell incubator at 37°C.

Cytotoxicity Measurements

The cytotoxicity of PMMA-*b*-P(GATH-*co*-BOD) nanoparticles against MCF-7 and RWPE-1 cells was evaluated by MTT assay. MCF-7 and RWPE-1 cells were seeded

with a density of 1.0×10^4 cells/well in 96-well plates cultured with 200 μL DMEM and K-SFM medium respectively. After incubation for 24 h in cell incubator at 37°C and 5% CO_2, the new medium and different concentration nanoparticles were added to replace the old one. Continuing to incubate for 48 h, the 20 μL of MTT solution(5 mg/ mL) in PBS was added to each well. Continuing to culture for another 4 h, the medium of every well was replaced by 200 μL DMSO, and the solution absorbance was measured by a BioTek Synergy H4 at 490 nm wavelength.

Cell Imaging

MCF-7 and RWPE-1 cells were seeded in cell culture dish at a density of 2.0×10^5 cells per well. 50 μg/mL PMMA-*b*-P(GATH-*co*-BOD) nanoparticles were added to the dish for incubating 1.5 h at 37 °C and 5% CO_2 atmosphere. The cell nucleus dye hoechst 33342 was added for another 30min. Then the cell dishes were washed with PBS for 3 times, and the cell samples were observed and photograph by (Leica)/TCS SP8STED 3X Super-resolution Multiphoton Confocal Microscope.

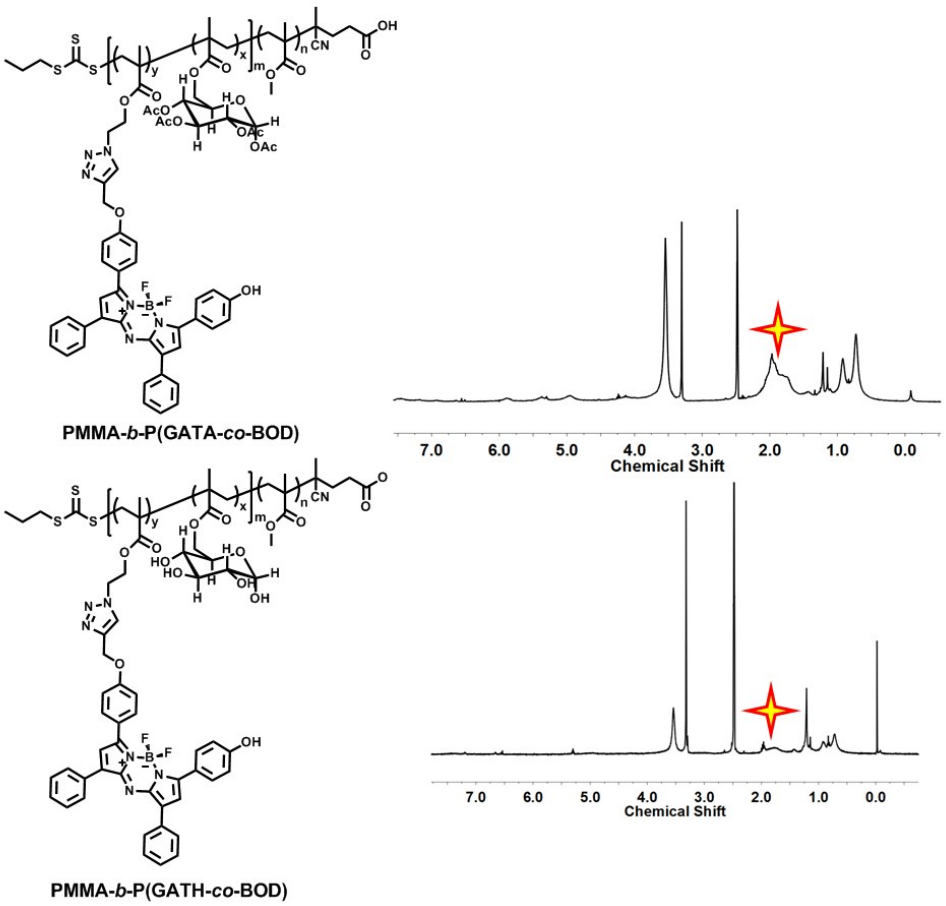


Figure S11. ^{1}H NMR spectra of deprotection for PMMA-*b*-P(GATA-*co*-BOD) inDMSO-d^{6}

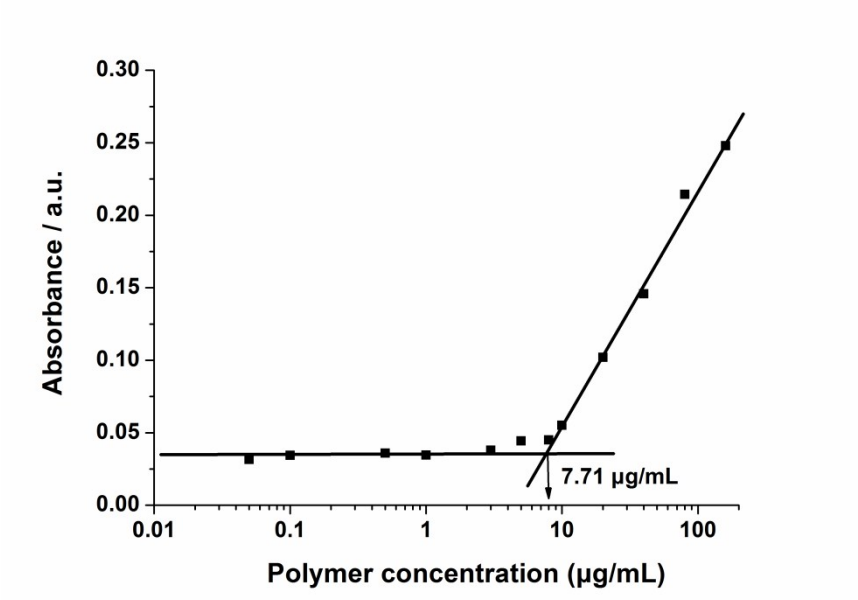


Figure S12. CMC of polymer PMMA-*b*-P(GATH-*co*-BOD) in aqueous solutions determined by

UV/Vis. (λ = 313 nm, 25 °C).

		Mean (mV)	Area (%)	Width (mV)
Zeta Potential (mV): -10.9	Peak 1:	-10.9	100.0	4.60
Zeta Deviation (mV): 4.60	Peak 2:	0.00	0.0	0.00
Conductivity (mS/cm): 0.276	Peak 3:	0.00	0.0	0.00
Result quality : Good				

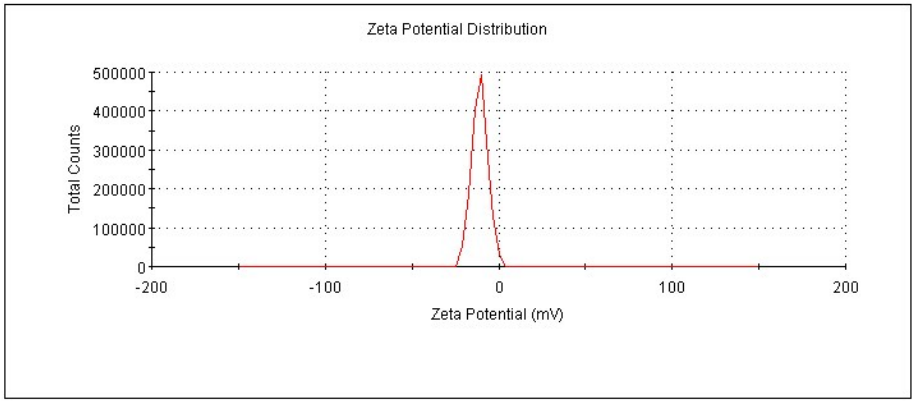


Figure S13. Zeta potential of polymer PMMA-*b*-P(GATH-*co*-BOD) micelles in aqueous solutions determined by DLS.

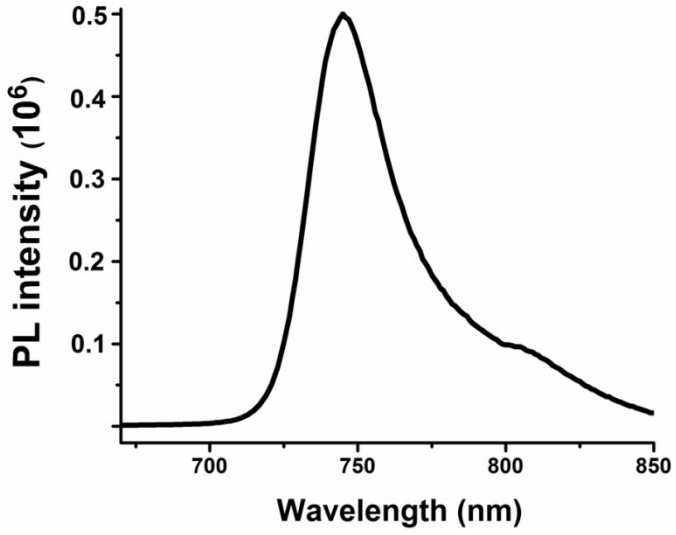


Figure S14. The emission spectra of fluorophore BOD-alky in DMSO, concentration = 5 μg/mL, λex=695 nm, λem=745 nm.

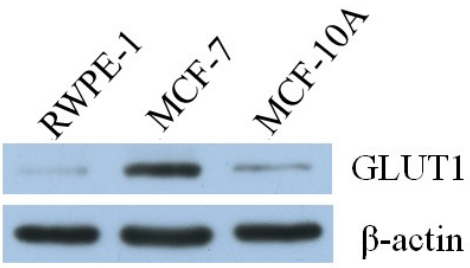


Figure S15. Western blot analysis of GLUT1protein in MCF-7, MCF-10A and RWPE-1cells

Table S1. monomers molar ratio of polymers PMMA and PMMA-*b*-P(GATA-*co*-BOD), and loading efficiency of BOD-alky

Polymer	CPP	MMA
PMMA	1	200

Polymer	PMMA	GATA	Et-N_3-MA	BOD-alky
PMMA-*b*-P(GATA-*co*-BOD)	1	25	2	1.48

Table S2. The quantum yields of polymer PMMA-*b*-P(GATH-*co*-BOD) in DMSSO at 25℃condition measured by integrating sphere of QM/TM/IM fluorescence Lifetime Spectrometer (PTI company).

Sample	Temperature	Solvent	Quantum yield
PMMA-*b*-P(GATH-*co*-BOD)	25℃	DMSO	0.09

Notes and references

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